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Synthesis and DNA Binding Properties of Iminodiacetic Acid-Linked Polyamides: Characterization of Cooperative Extended 2:1 Side-by-Side Parallel Binding

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Abstract: A series of iminodiacetic acid (IDA)-linked polyamides (DpPyPyPy-IDA-PyPyPyDp) were prepared and constitute polyamides joined head-to-head by a functionalizable five-atom linker. It was found that the IDA linker exerts a unique influence over the DNA binding conformation differing from both the β-alanine (extended) or γ-aminobutyric acid (hairpin) linkers, resulting in cooperative parallel side-by-side 2:1 binding in an extended conformation most likely with a staggered versus stacked alignment. A generalized variant of a fluorescent intercalator displacement (FID) assay conducted on a series of hairpin deoxyoligonucleotides containing a systematically varied A/T-rich binding-site size was used to distinguish between the binding modes of the IDA-linked polyamides.

Polyamides composed of *N*-methylpyrrole (Py), *N*-methylimidazole (Im), and a growing set of structural analogues bind in the DNA minor groove with predictable sequence selectivity and high affinities.[1] The head-to-tail linkage of such polyamides with the five-atom linker γ-aminobutyric acid (γ) has been shown to provide hairpin polyamides that mimic the 2:1 side-by-side antiparallel binding of the unlinked polyamides, enhance the binding affinity 10^2- to 10^4-fold, and improve the binding selectivity.[1a] In contrast, polyamides incorporating a one-carbon shorter head-to-tail linker, β-alanine (β), bind preferentially in an extended versus hairpin conformation forming 1:1 or side-by-side antiparallel 2:1 complexes.[2,3] Recently, we reported that appropriate substitution of a β-alanine linker can favor hairpin versus extended binding[4] providing an alternative linker to γ.[4] In continuation of studies on the impact of such polyamide linkers that might be easily assembled using solution-phase combinatorial techniques,[5] herein we report the synthesis and examination of the five-atom iminodiacetic acid (IDA)-linked polyamides. These efforts serve to complement and extend our preceding use of the IDA template in the solution-phase synthesis of combinatorial libraries[6] where the isolation and purification of intermediates and final products can be conducted using liquid−liquid or liquid−solid acid−base extractions.[5,6] In contrast to the head-to-tail polyamide linkage provided by γ and β, IDA provides a unique and unexplored head-to-head linkage through the N terminus of the pyrroles. Central to its structure is a functionalizable nitrogen capable of tethering additional DNA or protein binding or effector molecules including a simple dimerization of the polyamide that we felt might further extend its utility. The IDA structure and the resulting head-to-head linkage combine to provide an alternative binding mode differing from both the β-alanine (extended) and γ-aminobutyric acid (hairpin) linkers leading to cooperative parallel (versus antiparallel) side-by-side 2:1 binding in an extended conformation most likely with a staggered versus stacked alignment. This entails bidentate binding of the ligand (both halves) with one-half adopting a N-to-C/5′-to-3′ orientation and the other half adopting a N-to-C/3′-to-5′ orientation. Aside from the example of the indole-2,5-dicarboxylic linkage of the netropsin dipyrroles (GL020924) characterized by Bruice,[7] it represents only the second report of such head-to-head dimers binding in well-characterized cooperative 2:1 complexes, and as far as we can establish, it constitutes the first characterized example of parallel (versus antiparallel) side-by-side binding of such polyamides.

Just as importantly, we employed a generalizable variant of a recently disclosed fluorescent intercalator displacement (FID) assay[8] to study the DNA binding properties of **1**−**8** (Figure 1). Thus, the bound conformations of the IDA-linked polyamides

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(1) (a) Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215−2235. (b) Reddy, B. S. P.; Sondhi, S. M.; Lown, J. W. *Pharmacol. Ther.* **1999**, *84*, 1−111. (c) Neidle, S. *Nat. Prod. Rep.* **2001**, *18*, 291−309. (d) Bailly, C.; Chaires, J. B. *Bioconjugate Chem.* **1998**, *9*, 513−538.
(2) Trauger, J. W.; Baird, E. E.; Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6160−6166.
(3) Lamamie de Clairac, R. P.; Seel, C. J.; Geierstanger, B. H.; Mrksich, M.; Baird, E. E.; Dervan, P. B.; Wemmer, D. E. *J. Am. Chem. Soc.* **1999**, *121*, 2956−2964.
(4) Woods, C. R.; Ishii, T.; Wu, B.; Bair, K. W.; Boger, D. L. *J. Am. Chem. Soc.* **2002**, *142*, 2148−2152.
(5) Boger, D. L.; Fink, B. E.; Hedrick, M. P. *J. Am. Chem. Soc.* **2000**, *122*, 6382−6394.
(6) Cheng, S.; Comer, D. D.; Williams, J. P.; Boger, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 2567−2573. Cheng, S.; Tarby, C. M.; Comer, D. D.; Williams, J. P.; Caporale, L. H.; Boger, D. L. *Bioorg. Med. Chem.* **1996**, *4*, 727−737. Boger, D. L.; Chai, W.; Jin, Q. *J. Am. Chem. Soc.* **1998**, *120*, 7220−7225. Boger, D. L.; Chai, W. *Tetrahedron* **1998**, *54*, 3955−3970. Boger, D. L.; Goldberg, J.; Jiang, W.; Chai, W.; Ducray, P.; Lee, J. K.; Ozer, R.; Andersson, C.-M. *Bioorg. Med. Chem.* **1998**, *6*, 1347−1378. Boger, D. L.; Goldberg, J. In *Combinatorial Chemistry: A Practical Approach*; Fenniri, H., Ed.; Oxford University Press: Oxford, 2000; pp 303−326.
(7) Zhang, W.; Dai, Y.; Schmitz, U.; Bruice, T. W. *FEBS Lett.* **2001**, *509*, 85−89.

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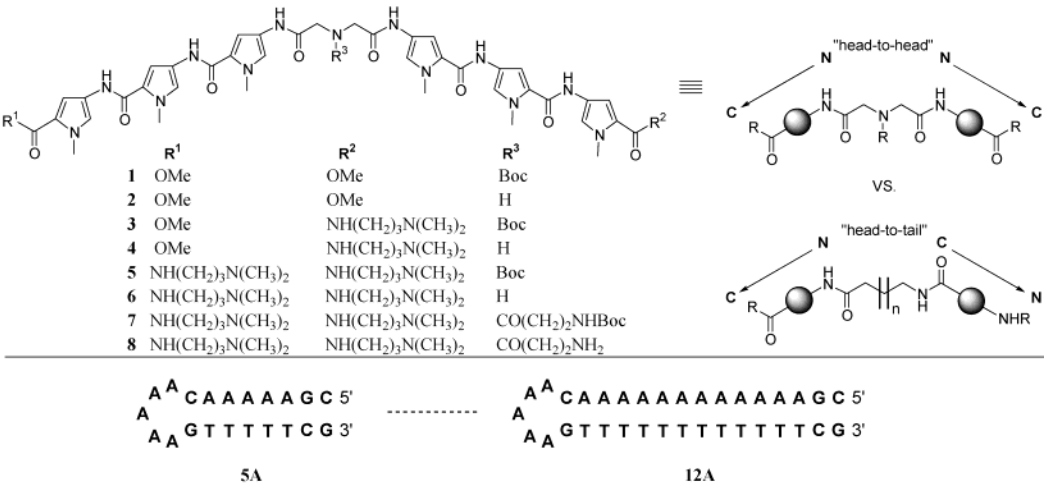
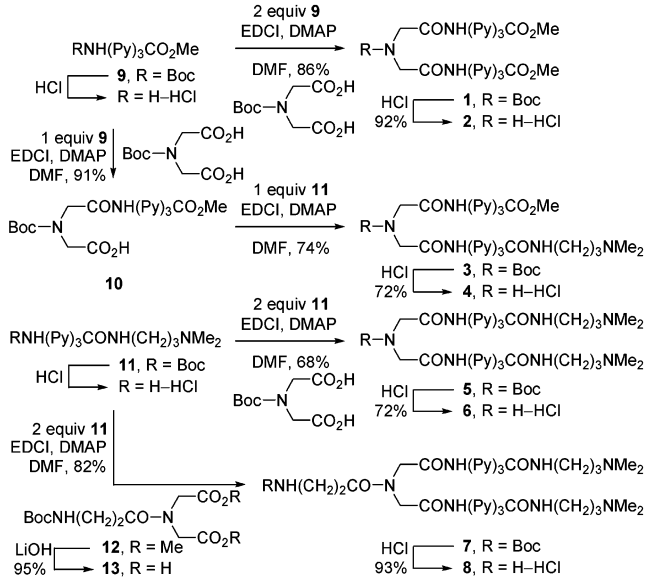


Figure 1. IDA-linked polyamides **1**−**8**. A/T-rich hairpin deoxyoligonucleotides containing 5−12 bp binding sites.

could be established by analysis[8,9] of FID titrations of hairpin deoxyoligonucleotides containing a systematically varied A/T-rich binding-site size.[4] Complementary assessments using a combination of footprinting and affinity cleavage techniques[10] are technically more demanding, require the separate preparation of the linked Fe-EDTA affinity cleavage derivatives, and do not as easily distinguish between such alternative binding modes.

Synthesis. The solution-phase synthesis of the polyamides was performed using a series of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI)-mediated coupling reactions as described previously where workup, isolation, and purification could be addressed principally by liquid−liquid acid−base extractions[5] (Scheme 1). *N*-Boc deprotection (HCl−EtOAc) of BocNH-PyPyPy-CO_2Me (**9**, 2 equiv),[11] followed by coupling with *N*-Boc-iminodiacetic acid (EDCI, DMAP, DMF, 25 °C, 86%) provided **1** (Scheme 1), which was subsequently *N*-Boc deprotected (HCl−EtOAc, 92%) to yield **2**. Similarly, *N*-Boc deprotection (HCl−EtOAc) of **9**[11] (1 versus 2 equiv), followed by coupling with *N*-Boc-iminodiacetic acid (EDCI, DMAP, DMF, 25 °C, 91%) provided **10**. *N*-Boc deprotection of BocNH-PyPyPy-CONH$(CH_2)_3$N$(CH_3)_2$ (**11**)[12] (HCl−EtOAc) and coupling of the free amine with **10** (EDCI, DMAP, DMF, 25 °C, 74%) provided **3**, which was subsequently *N*-Boc deprotected (HCl−EtOAc, 72%) to yield **4**. *N*-Boc deprotection (HCl−EtOAc) of **11** (2 equiv),[12] followed by coupling with *N*-Boc-iminodiacetic acid (EDCI, DMAP, DMF, 25 °C, 68%) provided **5**, which was subsequently *N*-Boc deprotected (HCl−EtOAc, 72%) to yield **6**. Coupling of BocNH$(CH_2)_3CO_2$H with dimethyl iminodiactetate (EDCI, DMAP, DMF, 25 °C, 93%) yielded diester **12** which was hydrolyzed (LiOH, THF−MeOH−H_2O, 95%) to yield diacid **13**. *N*-Boc deprotection (HCl−EtOAc) of **11** (2 equiv)[12] and coupling of the resulting free amine with diacid **13** (EDCI, DMAP, DMF, 25 °C, 82%) provided **7** which was subsequently *N*-Boc deprotected (HCl−EtOAc, 93%) to yield **8**.

Scheme 1



Preliminary Screening and Assessment of the Impact of the Charged End Groups on DNA Binding. The importance of the charged *N*,*N*-dimethylaminopropyl (Dp) end groups was first established by examining compounds **2**, **4**, and **6** alongside distamycin and **15** for DNA binding to an A/T-rich hairpin DNA containing a 5 bp binding site using the FID assay (Figure 2). Thus, the relative binding affinities were established by monitoring the loss of fluorescence derived from titration displacement of prebound ethidium bromide from the DNA. A comparison the compound concentration required for 50% displacement of the ethidium bromide (C_{50}) revealed that only **6**, containing two charged Dp end groups, showed significantly greater binding affinity than distamycin. In fact, the C_{50} trend with **6** (2 Dp's) > **4** (1 Dp) > **2** (no Dp's) was clear, indicating that the presence of the Dp end group is an important factor in DNA binding. Whereas compound **2** was relatively ineffective

(8) Boger, D. L.; Fink, B. E.; Brunette, S. R.; Tse, W. C.; Hedrick, M. P. *J. Am. Chem. Soc.* **2001**, *123*, 5878−5891. Boger, D. L.; Tse, W. C. *Bioorg. Med. Chem.* **2001**, *9*, 2511−2518.

(9) (a) Scatchard, G. *N.Y. Acad. Sci.* **1949**, *51*, 660−672. (b) Perkins, H. R. *Biochem. J.* **1969**, *111*, 195−205. (c) Schmitz, H.-U.; Hübner, W. *Biophys. Chem.* **1993**, *48*, 61−74.

(10) Footprinting: (a) Dervan, P. B. *Science* **1986**, *232*, 464−471. (b) Galas, D. J.; Schmitz, A. *Nucleic Acids Res.* **1978**, *5*, 3157−3170. (c) Royer-Pokora, B.; Gordon, L. K.; Haseltine, W. A. *Nucleic Acids Res.* **1981**, *9*, 4595−4609. (d) Van Dyke, M. W.; Hertzberg, R. P.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5470−5474. (e) Tullius, T. D.; Dombrowski, B. A.; Churchill, M. E. A.; Kam, L. *Methods Enzymol.* **1987**, *155*, 537−538. (f) Kuwabara, M.; Sigman, D. S. *Biochemistry* **1987**, *26*, 7234−7238. Affinity cleavage: (g) Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457−465.

(11) Bailly, C.; Pommery, N.; Houssin, R.; Henichart, J. P. *J. Pharm. Sci.* **1989**, *78*, 910−917.

(12) Bailly, C.; Pommery, N.; Houssin, R.; Henichart, J. P. *J. Pharm. Sci.* **1989**, *78*, 910−917.

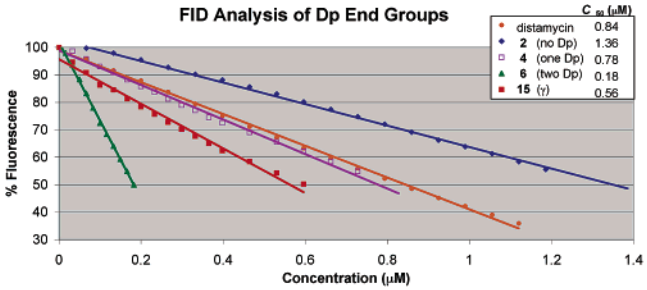


Figure 2. FID titrations and C_{50} values of distamycin, **2**, **4**, **6**, and **15**.

Table 1. Binding of **3**, **5**−**8**, and **14**−**16** to Hairpin Deoxyoligonucleotides Containing 5−12 bp A/T-Rich Sites[a]

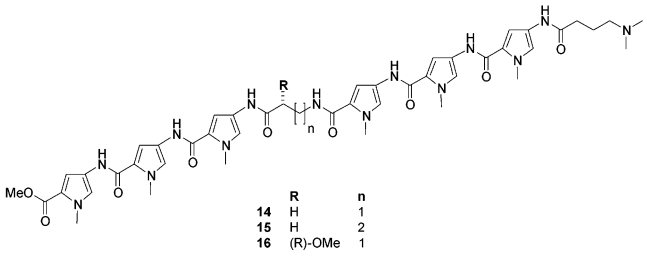
A/T-length	3		5		6		8	
(bp)	stoich.	K^b	stoich.	K^b	stoich.	K^b	stoich.	K^b
5	1.9	6.9	1.6	9.3	1.6	4.5	2.0	15
6	1.6	5.6	1.8	16	1.9	12	2.0	14
7	2.0	9.9	1.6	16	1.9	19	2.0	15
8	1.8	12	1.6	13	2.0	18	2.0	14
9	2.0	19	1.9	28	2.0	20	2.0	22
10	1.5	19	2.0	29	2.0	20	2.0	21
11	1.7	13	2.0	27	2.0	27	2.0	20
12	1.8	15	2.0	27	2.0	29	2.0	20

A/T-length	7		14		15		16	
(bp)	stoich.	K^b	stoich.	K^b	stoich.	K^b	stoich.	K^b
5	1.6	11	1.5	1.4	1.0	5.5	1.0	8.4
6	1.6	17	1.5	2.8	1.0	7.3	1.0	9.1
7	1.5	17	1.5	1.9	1.0	6.7	1.0	8.6
8	1.9	34	1.9	120	1.1	6.0	1.0	6.4
9	1.9	44	1.9	130	1.1	13	1.0	8.2
10	2.0	46	1.9	130	1.7	17	1.0	8.0
11	2.0	50	2.0	200	2.0	24	2.0	9.5
12	2.0	53	2.0	190	2.0	27	2.0	7.1

[a] Average of triplicate determination, stoichiometry, and K (<±5%). [b] Values × 10^7 M^{-1}.

and **4** was essentially indistinguishable from distamycin itself, compound **6** was found to be an effective DNA binding agent exhibiting an affinity for the hairpin DNA that exceeded that of not only distamycin but also the γ-hairpin polyamide **15**. With these initial observations, our studies were expanded to an examination of a range of IDA-linked polyamides (**3**−**8**) containing one and two Dp end groups.

Distinguishing Extended versus Hairpin Binding of Polyamides. For comparison, the β and γ polyamides **14**[4] and **15** were examined alongside **1**−**8** (Tables 1 and 2 and Figures 3 and 4). The β-alanine-linked polyamide **14** binds in a 2:1 complex in the extended conformation over an 8 bp A/T-rich site. Thus, effective binding constants for **14** are observed only with the DNA containing A/T-rich sites ≥8 bp and the experimental stoichiometry is 2:1 reflective of cooperative side-by-side antiparallel binding adopting the preferred N-to-C/5′-to-3′ orientation.[13] By contrast, the γ-aminobutyric acid-linked polyamide **15** binds effectively to the DNAs containing even the short A/T-rich sites adopting a hairpin conformation and a 1:1 binding stoichiometry. This binding affinity and stoichiometry remain constant over the binding-site sizes of 5−8/9 bp and then increases to 2:1 binding effectively only for the longer 11−12 bp sites. This is consistent with binding of two adjacent γ-polyamides, both bound in hairpin conformations and each binding a 5−6 bp A/T-rich site. As discussed below, the number of intercalators displaced throughout the series of DNA hairpins can oftentimes be used to independently confirm the binding-site size and hence the bound conformation of the polyamide.



A third comparison with the polyamide **16**[4] containing the (*R*)-α-methoxy-β-alanine linker ($\beta^{(R)-OMe}$) represents an even better example of polyamide hairpin binding in this assay. The binding affinity of **16** for the 5−12A DNA remains unchanged throughout the series, and the stoichiometry increases from 1:1 (5−10A) to 2:1 (11−12A) once the binding-site size is large enough to accommodate two adjacent hairpin-bound polyamide conformations, each requiring a 5A site. In fact, the comparison of the binding constant changes through the 5A−12A DNA series for **15** with **16** versus **14** suggests that the γ-polyamide **15** may bind the longer sequences as an extended 2:1 side-by-side antiparallel dimer like the β-polyamide **14** rather than as two adjacent hairpins like **16**.

Binding of IDA-Linked Polyamides to Hairpin Deoxyoligonucleotides Containing Variable-Length A/T-Rich Binding Sites. The comparisons of the doubly charged IDA derivatives **5**−**8** and the singly charged **3** were made using the FID titration assay with the series of DNA hairpins where the A/T-rich binding site was varied from 5 to 12 bp.[14] Comparing the behavior of **5**−**8** as the length of the binding region is increased, the stoichiometry of binding ranges from 1.6 to 2.0 for 5−8 bp and then increases to 2 for 9−12 bp. Similarly, the binding constants increase with the longer 9−12 bp binding regions compared with the shorter 5−8 bp sequences. With **5**, **6**, and **8**, this increase in binding constant is moderate (ca. 2- to 3-fold). However, the increase is larger with **7** (ca. 3- to 5-fold). With compound **3**, containing only one charged Dp end group, the stoichiometry of binding fluctuates from 1.5 to 2, and it displays binding constants consistently weaker than those for **5**−**8**. Compound **3** also experiences a moderate, although inconsistent, increase in binding constant as the binding site gets larger (ca. 2-fold).

This behavior is inconsistent with **3** and **5**−**8** adopting bound hairpin conformations like that of **16**. It is much more consistent with the bidentate behavior of **14** adopting an extended bound conformation and cooperative 2:1 binding. Consistent with the initial observations, two charged end groups > one charged end group > no charged end group (**7** vs **3**). Interestingly and although the sampling of potential structures is too small to draw

(13) Details of the FID assay development, the technique used to establish the stoichiometry of binding (intersection of pre- and post-saturation curves in titration), the technique used to establish K (Scatchard analysis of titration curve), comparisons of the accuracy of binding constants established using the indirect measurement of the ethidium bromide displacement versus direct fluorescent measurements of binding agents (≤2-fold difference), and studies establishing that the accuracy of the measurements are not sequence-dependent may be found in ref 8.

(14) The exclusion of **4** in these assessments simply reflects the fact that it was no longer available at the time the hairpin 5−12A screens became available.

Table 2. Number of Ethidium Bromide Molecules Displaced upon Binding of **3**, **5**−**8** and **14**−**16** to Hairpin Deoxyoligonucleotides Containing 5−12 bp A/T-Rich Sites

		3		**5**		**6**		**8**	
A/T-length (bp)	number of EtBr bound	$F/F_{100\%}$[a]	EtBr displaced ($F_t/F_{100\%}$)[b]	$F/F_{100\%}$[a]	EtBr displaced ($F_t/F_{100\%}$)[b]	$F/F_{100\%}$[a]	EtBr displaced ($F_t/F_{100\%}$)[b]	$F/F_{100\%}$[a]	EtBr displaced ($F_t/F_{100\%}$)[b]
5	4	0.66	1 (0.75)	0.40	2 (0.50)	0.53	2 (0.50)	0.46	2 (0.50)
6	4	0.71	1 (0.75)	0.44	2 (0.50)	0.45	2 (0.50)	0.45	2 (0.50)
7	5	0.68	2 (0.60)	0.51	3 (0.40)	0.40	3 (0.40)	0.47	3 (0.40)
8	5	0.66	2 (0.60)	0.53	2 (0.60)	0.44	3 (0.40)	0.50	3 (0.40)
9	6	0.75	2 (0.67)	0.53	3 (0.50)	0.50	3 (0.50)	0.58	3 (0.50)
10	6	0.76	2 (0.67)	0.49	3 (0.50)	0.51	3 (0.50)	0.62	2 (0.67)
11	7	0.77	2 (0.71)	0.48	4 (0.43)	0.53	3 (0.57)	0.60	3 (0.57)
12	7	0.76	2 (0.71)	0.46	4 (0.43)	0.53	3 (0.57)	0.61	3 (0.57)
		7		**14**		**15**		**16**	
A/T-length (bp)	number of EtBr bound	$F/F_{100\%}$[a]	EtBr displaced ($F_t/F_{100\%}$)[b]	$F/F_{100\%}$[a]	EtBr displaced ($F_t/F_{100\%}$)[b]	$F/F_{100\%}$[a]	EtBr displaced ($F_t/F_{100\%}$)[b]	$F/F_{100\%}$[a]	EtBr displaced ($F_t/F_{100\%}$)[b]
5	4	0.34	3 (0.25)	0.41	2 (0.50)	0.34	3 (0.25)	0.27	3 (0.25)
6	4	0.41	2 (0.50)	0.29	3 (0.25)	0.34	3 (0.50)	0.34	3 (0.25)
7	5	0.42	3 (0.40)	0.27	4 (0.20)	0.44	3 (0.40)	0.40	3 (0.40)
8	5	0.52	2 (0.60)	0.30	4 (0.20)	0.47	3 (0.40)	0.44	3 (0.40)
9	6	0.54	3 (0.50)	0.38	4 (0.33)	0.46	3 (0.50)	0.45	3 (0.50)
10	6	0.50	3 (0.50)	0.40	4 (0.33)	0.43	3 (0.50)	0.45	3 (0.50)
11	7	0.49	4 (0.43)	0.44	4 (0.43)	0.35	5 (0.38)	0.20	6 (0.14)
12	7	0.47	4 (0.43)	0.46	4 (0.43)	0.32	5 (0.38)	0.17	6 (0.14)

[a] $F/F_{100\%}$ is the experimentally derived ratio of remaining fluorescence. [b] $F_t/F_{100\%}$ is the theoretical ratio of remaining fluorescence after the indicated number of ethidium bromide (EtBr) molecules have been displaced.

substantive conclusions from, a free amino group within the linker (**6** vs **5**) or attached to the linker (**8** vs **6**) did not improve binding affinity and was detrimental in the latter comparison. Thus, both charged end groups of **5**−**8** contribute productively to their binding behavior, but the first has a more substantial effect than the second. Moreover, it further supports a bidentate versus monodentate binding mode for the doubly charged compounds. N-acylation of the iminodiacetic acid linker does not adversely effect the binding (**5** and **8** ≈ **6**, NBoc and NCO-$(CH_2)_3NH_2$ vs NH). Most significantly, one of the *N*-acyl derivatives, **7** ($NCO(CH_2)_3NHBoc$), exhibited higher binding constants than that of **6** and a pattern of binding affinity/stoichiometry that most closely parallels that of *β*-linked polyamides adopting a cooperative 2:1 side-by-side extended binding. For **5** and **7**, the best behaved compounds in the series, the full stoichiometry of binding (2:1) and affinity were reached with an A/T-rich binding-site size of 9−10 bp consistent with cooperative, extended 2:1 side-by-side binding covering a 9−10 bp site. It is notable that for even the shorter 5−8 bp AT-rich sites, cooperative but nonoptimal 2:1 binding is observed for all the compounds which we have interpreted to simply represent 2:1 complexes of partial bound polyamides potentially adopting an antiparallel arrangement covering 5−6 bp.

Bound Conformation. Comparison of the agent binding stoichiometry with the number of ethidium bromide molecules displaced upon binding can provide additional information on the binding-site size and bound conformation of the flexible molecules. However, exceptions to this have been noted (i.e., netropsin > distamycin),[8] suggesting caution should be used in interpreting such results. The number of ethidium bromide molecules displaced can be approximated by examining the fluorescence value for DNA saturated with ethidium bromide ($F_{100\%}$) versus the final fluorescence value after the titration is complete and the DNA is saturated with agent (F). Assuming ethidium bromide intercalates at a ratio of one molecule per two bp of DNA, the ratio of $F/F_{100\%}$ can be compared to the theoretical fluorescence decrease (F_t) expected for any stoichiometry of ethidium bromide displacement (Figure 5).

The number of ethidium bromides displaced by **14**[4] most closely approximates the constant value of 4 throughout elongation of the A/T binding site from 7 to 12 bp consistent with antiparallel 2:1 side-by-side binding across an 8 bp site (Table 2). For **15** and **16**,[4] the first binding event displaces three ethidium bromides and is observed without change throughout the 5−10 bp binding-site size range. The second binding event displaces an additional two to three ethidium bromides and is only observed with the longer sequences (11 and 12 bp). Since the stoichiometry of binding for the first event is one and the latter is two, this implies two consecutive binding events of **15** and **16** adopting a hairpin conformation, each requiring non-overlapping 5−6 bp A/T-rich sites. For the two best behaved compounds in the IDA series, **5** and **7**, the number of intercalators displaced steadily increases to four through the 5−12A series, indicating a binding-site size of 8 or 9 bp. This proved consistent with the observation that their cooperative 2:1 stoichiometry of binding and binding constants also require a minimum of 9−10 bp for full binding.

Molecular modeling of the **5**−**8** indicates that an extended, side-by-side, fully overlapping conformation would span approximately 9−10 bp. However, this model of extended 2:1 binding requires parallel, not antiparallel, side-by-side binding of the polyamides and that one-half of each structure would be binding in the N to C/3′ to 5′ orientation. Whereas the parallel binding might be accommodated by a staggered versus stacked arrangement of the polyamides, the adoption of a nonpreferred bound orientation for one-half of each molecule would not appear to be optimal. However, the preference for binding orientation is related to the nature of both the head and tail end groups. Thus, whereas distamycin exhibits a characteristic N-to-C/5′- to-3′ binding orientation attributable to the *N*-formyl/*C*-propylamidine substitutions, netropsin does not, due to its near symmetrical *N*- and *C*-amidine functionalization.[15] Additionally,

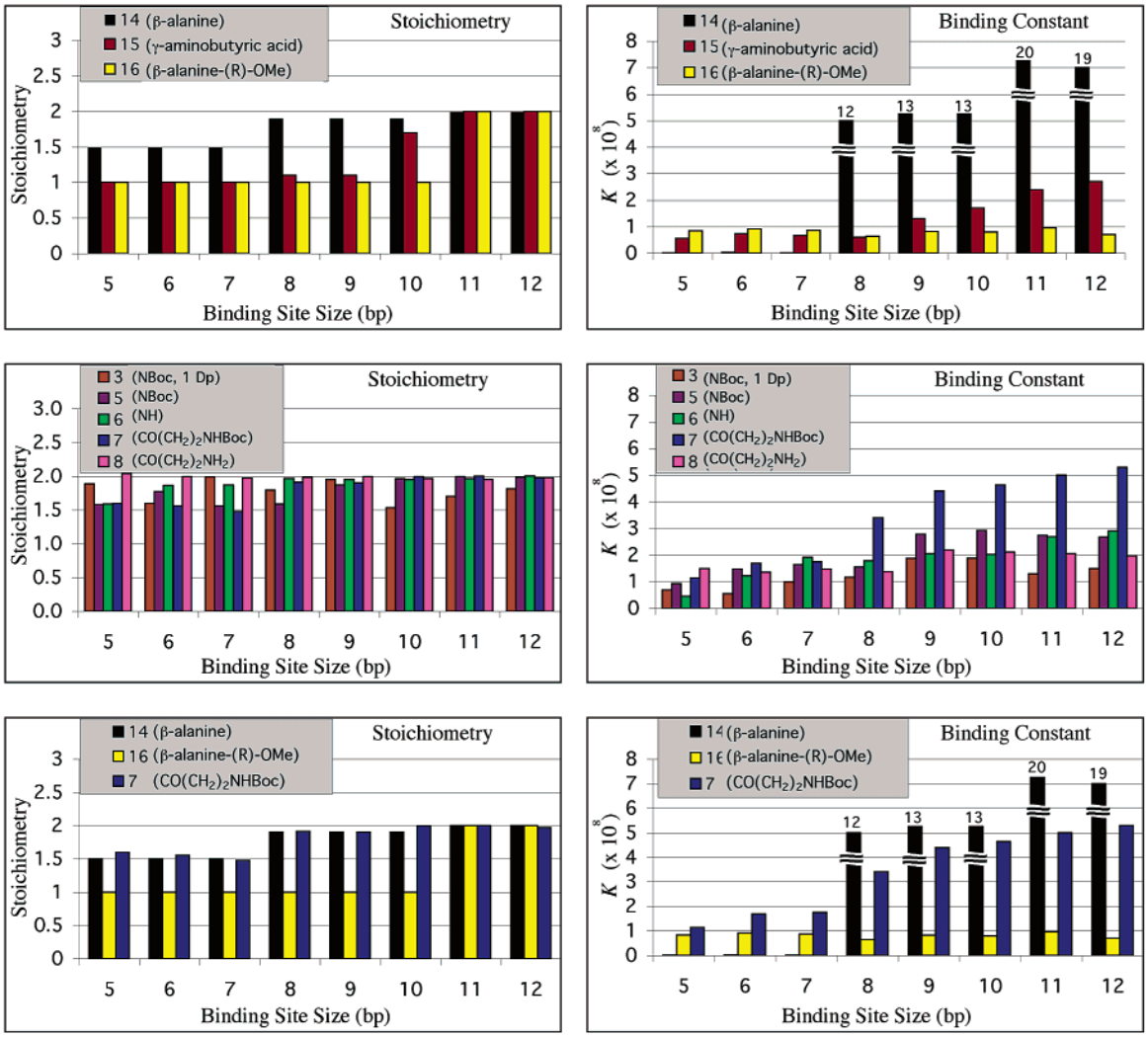


Figure 3. Comparison of stoichiometry and binding constants of **3**, **5**−**8**, and **14**−**16**.

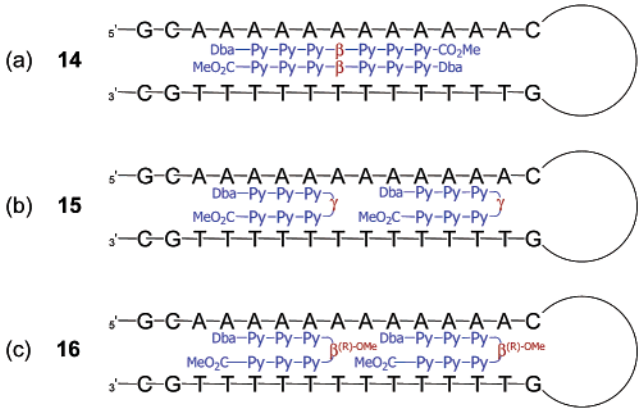


Figure 4. Representation of (a) **14**, (b) **15**, and (c) **16** binding to A/T-rich hairpin DNA containing a 12 bp binding region.

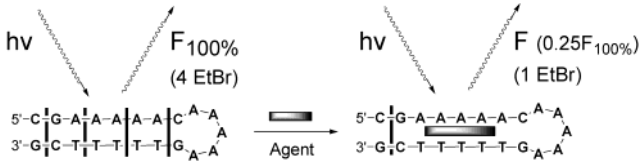


Figure 5. Reduction in ethidium bromide fluorescence from saturated ($F_{100\%}$) occurs upon binding by agent, yielding a final state (F) where three of four intercalated ethidium bromide molecules, shown as (|), have been displaced from the 5 bp A/T-rich hairpin deoxynucleotide ($F = 0.25F_{100\%}$).

Wemmer and Dervan have defined features of the polyamides that contribute to the orientational binding and have observed reversed N-to-C/3′-to-5′ binding with selected hairpin polyamides.[16] In part, this may be attributable to the C-terminus charged group effects which prefer, but do not require the N-to-C/5′-to-3′ binding, and N-acylation with groups other than formyl which reinforces a reversed versus natural binding orientation of distamycin. The doubly end charged nature of compounds including **7** (like netropsin) coupled with their N-acylation through the IDA linker appears to alter the binding orientation preference such that they may adopt both the normal or reversed modes required for the extended binding of **7**. The most unique behavior of **7** is that this then leads to parallel, not antiparallel, cooperative 2:1 side-by-side binding which, to our knowledge, has not been previously characterized. A possible

(15) Pelton, J. G.; Wemmer, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5723−5727. Pelton, J. G.; Wemmer, D. E. *J. Am. Chem. Soc.* **1990**, *112*, 1393−1399. Fagan, P. A.; Wemmer, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 1080−1081. Fagan, P. A. Ph.D. Thesis, University of California Berkeley, 1996.

(16) Hawkins, C. A.; de Clairac, R. P.; Dominey, R. N.; Baird, E. E.; White, S.; Dervan, P. B.; Wemmer, D. E. *J. Am. Chem. Soc.* **2000**, *122*, 5235−5243.

Table 3. Binding of **6** and **7** to Hairpin Deoxyoligonucleotides Containing 11 bp Binding Sites[a]

	6				**7**			
DNA sequence 5'to 3' 	Stoich.	K ($\times 10^7$ M^{-1})	$F/F_{100\%}$[a]	EtBr displaced $(F_t/F_{100\%})$[b]	Stoich.	K ($\times 10^7$ M^{-1})	$F/F_{100\%}$[a]	EtBr displaced $(F_t/F_{100\%})$[b]
aaaaaAaaaaa	2.0	27	0.52	3 (0.57)	2.0	50	0.49	4 (0.43)
aaaaaTaaaaa	2.0	25	0.63	3 (0.57)	1.9	28	0.34	5 (0.38)
aaaaaCaaaaa	2.0	25	0.62	3 (0.57)	2.0	5.2	0.49	4 (0.43)
aaaaaGaaaaa	2.0	24	0.61	3 (0.57)	1.9	9.5	0.64	3 (0.57)
aaaaaGGaaaa	2.0	23	0.73	2 (0.71)	2.0	8.6	0.59	3 (0.57)
aaaaaGGGaaa	2.0	3.2	0.71	2 (0.71)	1.9	3.0	0.64	3 (0.57)
aaaaaGGGGaa	2.0	9.7	0.70	2 (0.71)	1.9	6.8	0.68	2 (0.71)

[a] Average of triplicate determination, stoichiometry, and K ($<\pm 5\%$). [b] $F/F_{100\%}$ is the experimentally derived ratio of remaining fluorescence. [c] $F_t/F_{100\%}$ is the theoretical ratio of remaining fluorescence after the indicated number of ethidium bromide (EtBr) molecules have been displaced.

alternative conformation is one in which the opposite halves of the molecules overlap in an antiparallel 2:1 fashion in the center of the complex, and the other polyamide halve(s) extend in a 1:1 manner along the minor groove in opposite directions or lie outside the minor groove. The former complexes would require optimal binding-site sizes that substantially exceed ($\geq$12−14 bp) those observed with **7** (9−10 bp), and the latter would appear to be at least as accessible or better for singly charged compounds such as **3** versus **7**. Thus, while this may well represent a competing mode of binding, it is not consistent with the binding behavior of **7** toward the 9−12 bp A/T sites.

Additional DNA Sequences. In efforts to further distinguish the bound conformations of the IDA-linked derivatives, the FID titration analysis of **6** and **7** was explored with 11 bp A/T-rich hairpin deoxyoligonucleotides containing T/A, C/G, and G/C substitutions central to the A/T site, Table 3. For **6**, no change in behavior was seen in stoichiometry, binding constant, or number of ethidium bromide molecules displaced for sequences with a single, central bp substitution (T, C, or G vs A). Similarly, incorporation of two adjacent GC bps central to the site had no impact on the behavior of **6** although the number of displaced ethidium bromide intercalators decreased, whereas incorporation of three and four GC bp led to a 10-fold decrease in binding affinity consistent with disrupted extended bidentate binding. This suggests bidentate extended binding of the ligand with the IDA linker spanning up to two GC bp. Compound **7**, the highest affinity and best-behaved binding ligand in the series, exhibited a greater sensitivity to these same sequence changes. Substitution of the central base pair with C or G (10-fold), and to a minor extent T (<2-fold), led to reductions in binding affinity that remained relatively unchanged or were further reduced with the subsequent GG, GGG, and GGGG substitutions. This again is consistent with bidentate-extended binding of the ligand with the N-acylated IDA linker spanning only AT bps. Thus, as the number of substitutions increases from the central bp toward the 3′ end (G, GG, GGG, and GGGG), the binding affinity of **6** is constant with 0−2 G, then reduces with 3 G and 4 G, whereas the binding constant for **7** is reduced and stays approximately the same for the 1−4 G substitutions.

One interpretation of this behavior is that **6** adopts an extended bound conformation with the IDA linker spanning up to two central base pairs without exhibiting a selectivity (AA = TA, CA, GA, GG). Full extended binding is disrupted with the GGG and GGGG substitutions representing occluded minor groove binding of one-half of the compound. By contrast, the most effective agent in the series (**7**) is much more sensitive to these changes at the center of the sequence. Thus, the 2:1 extended binding of the N-acylated IDA-linked polyamides requires an A or T bp and is disrupted by an intervening GC bp. This suggests that the unsubstituted IDA linker and its free basic nitrogen with **5** may simply sterically accommodate up to two GC bp or that it may H-bond with and accommodate G. Although our studies do not distinguish such possibilities, a slipped side-by-side parallel binding with two IDA/Py pairings may well represent the binding spanning two adjacent G's (GG). Acylation on nitrogen as with **7** could disrupt either of these capabilities and limit the IDA binding to spanning only an A or T.

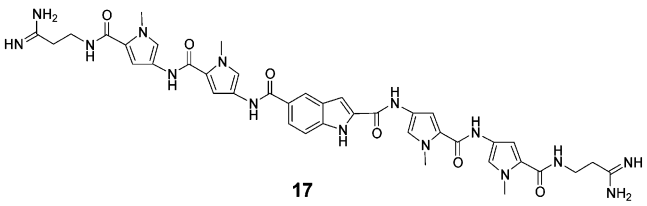
Discussion

The presence of the IDA linker has a unique effect on the ability of the polyamides to bind the DNA minor groove and the mode in which it binds. Unlike the head-to-tail β-alanine linkers that result in the formation of an extended[2] or hairpin[4]-bound conformation of the polyamide or the head-to-tail γ-aminobutyric acid linker[1a] which induces a hairpin-bound conformation of the polyamide, the IDA linker provides a different binding mode. For **5** and **7**, the best-behaved compounds in the series, cooperative extended 2:1 binding across a 9−10 bp site is observed with the IDA-linked ligands in what appears to be the first well-characterized example of parallel, versus antiparallel, side-by-side binding of such polyamides.

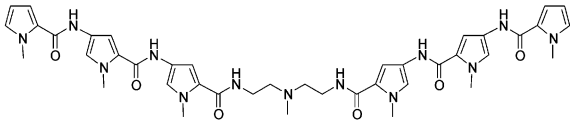
Recently, polyamide **17** was characterized by Bruice[7] which is linked head-to-head by a central 2,5-indole dicarboxylic acid unit. Ligand **17** (GL020924) is the only other well-characterized head-to-head linked polyamide shown to bind cooperatively in a 2:1 complex, whereas other prior examples that have been characterized bind as 1:1 complexes with both monodentate and bidentate binding or exhibit ill-characterized mixed modes of binding.[17] Moreover, **17** (which is two pyrrole units shorter than **7**) has been proposed to represent partially overlapped antiparallel side-by-side binding spanning up to 12 A/T bp. Such a complex for **5** and **7** would require 14 bp for full binding, unlike that which is experimentally observed at 9−10 bp. Similarly, the recent report of the first novel tail-to-tail linked polyamide **18**[18] was disclosed, and it was found to exhibit bidentate binding and stoichiometries for binding to poly[dAT] consistent with 2:1 versus 1:1 binding. This was interpreted to represent the alternative partially overlapped antiparallel side-by-side binding that leads to alternating "multimeric binding" with each ligand

assumed to cover 8−10 bp (each dimer covering ∼14 bp). Whereas we cannot rule out that such complexes may be available with **5** and **7** if examined in longer A/T-rich sequences, the experimental behavior of **5** and **7** within the 5−12A binding sites is most consistent with cooperative, parallel 2:1 side-by-side binding across a 9−10 bp site.

Thus, the head-to-head IDA-linked polyamides provide unique DNA-bound complexes distinct from those observed with β-alanine (extended) or γ-aminobutyric acid (hairpin). Like β-alanine-linked polyamides, they adopt extended versus hairpin-bound conformations and exhibit cooperative 2:1 side-by-side binding. Unlike the β-alanine-linked polyamides which adopt antiparallel side-by-side binding, the IDA-linked polyamides necessarily adopt a complementary and previously uncharacterized parallel side-by-side binding. This, along with the functionalizable nitrogen central to the IDA linker, provides new paradigms for the design of sequence-selective DNA binding and effector ligands.



17



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Supporting Information Available: Characterization and experimental details for the preparation of **1**−**8** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Fishleigh, R. V.; Fox, K. R.; Khalaf, A. I.; Pitt, A. R.; Scobie, M.; Suckling, C. J.; Urwin, J.; Waigh, R. D.; Young, S. C. *J. Med. Chem.* **2000**, *43*, 3257−3266. Khalaf, A. I.; Pitt, A. R.; Scobie, M.; Suckling, C. J.; Urwin, J.; Waigh, R. D.; Fishleigh, R. V.; Young, S. C.; Wylie, W. A. *Tetrahedron* **2000**, 56, 5225−5239. Burckhardt, G.; Simon, H.; Storl, K.; Triebel, H.; Walter, A.; Lown, J. W.; Zimmer, C. *J. Biomol. Struct. Dyn.* **1997**, *15*, 81−95. Lown, J. W.; Krowicki, K.; Balzarini, J.; Newman, R. A.; DeClercq, E. *J. Med. Chem.* **1989**, *32*, 2368−2375. Rao, K. E.; Zimmerman, J.; Lown, J. W. *J. Org. Chem.* **1991**, *56*, 786−797. Griffin, J. H.; Dervan, P. B. *J. Am. Chem. Soc.* **1986**, *108*, 5008−5009. Youngquist, R. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1985**, *107*, 5528−5529. Schultz, P. G.; Dervan, P. B. *J. Am. Chem. Soc.* **1983**, *105*, 7748−7750. Wang, W.; Lown, J. W. *J. Med. Chem.* **1992**, *35*, 2890−2897. Singh, M. P.; Plouvier, B.; Hill, G. C.; Gueck, J.; Pan, R. T.; Lown, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 7006−7020. Kissinger, K. L.; Dabeowiak, J. C.; Lown, J. W. *Chem. Res. Toxicol.* **1990**, *3*, 162−168. Rao, K. E.; Krowczki, K.; Balzarini, J.; DeClercq, E.; Newman, R. A.; Lown, J. W. *Actual Chim. Ther.* **1991**, *18*, 21−42. Surovaya, A. N.; Burckhardt, G.; Grokhovsky, S. L.; Birch-Hirschfeld, E.; Nikitin, A. M.; Fritzche, H.; Zimmer, C.; Gursky, G. V. *J. Biomol. Struct. Dyn.* **2001**, *18*, 689−701. Grokhovsky, S. L.; Surovaya, A. N.; Burckhardt, G.; Pismensky, V. F.; Chernov, B. K.; Zimmer, C.; Gursky, G. V. *FEBS Lett.* **1998**, *439*, 346−350. Surovaya, A. N.; Burckhardt, G.; Grokhovsky, S. L.; Birch-Hirschfeld, E.; Gursky, G. V.; Zimmer, C. *J. Biomol. Struct. Dyn.* **1997**, *14*, 595−606. Sinyakov, A. N.; Ryabinin, V. A.; Grimm, G. N.; Boutorine, A. S. *Mol. Biol. (Russ) Engl. Tr.* **2001**, *35*, 251−260.

(18) Bhattacharya, S.; Thomas, M. *Chem. Commun.* **2001**, 1464−1465.